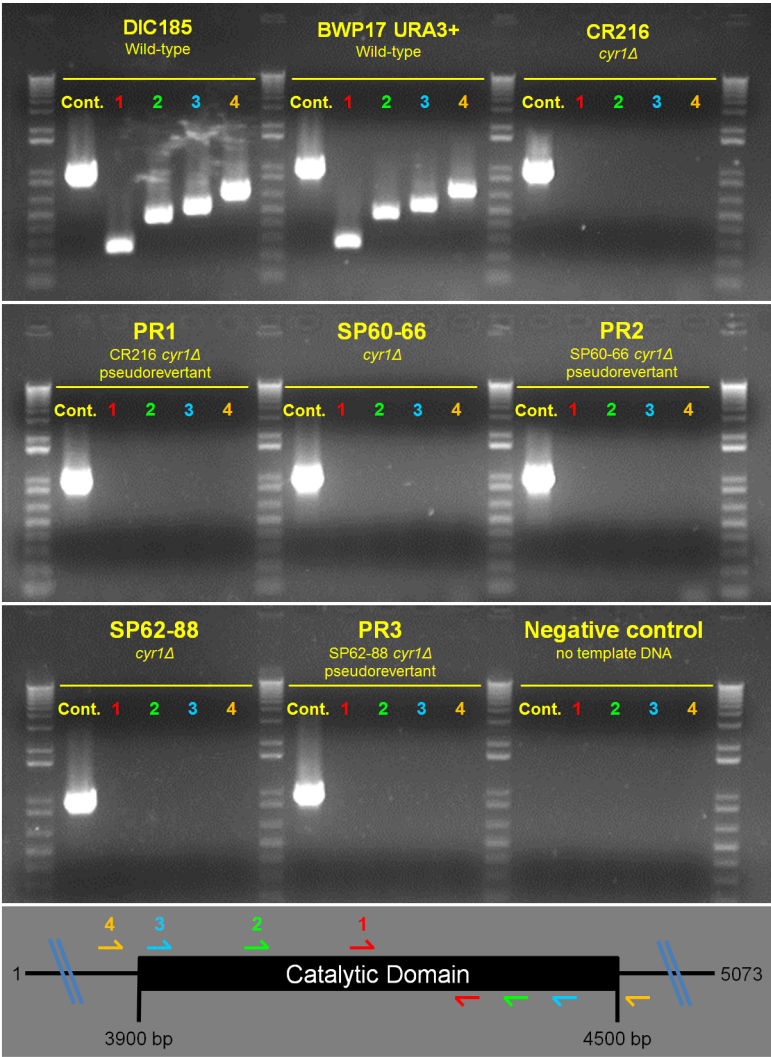


Supplementary Information



Supplemental Figure 1

Supplemental Figure 1: Pseudorevertants derived from *cyr1Δ* strains lack the *CYR1* catalytic domain.

The adenylyl cyclase gene *CYR1* contains a 5073 bp open reading frame, in which the catalytic domain that is essential for cAMP production occurs between 3900 and 4500 bp (bottom panel). To ensure that no remnants of the catalytic domain remained in the genomes of the *cyr1Δ* strains and their derived pseudorevertants, PCR analysis was carried out with different sets of primers homologous to the catalytic domain. The PCR products were then separated by electrophoresis on an agarose gel and detected by staining with ethidium bromide. All strains gave a positive PCR result for the control primers that amplified a section of the *BCY1* gene (lanes labeled Cont.). The wild type control strains (DIC185 and BWP17 URA3+) also gave the expected PCR products for four different sets of primers that span the catalytic domain of *CYR1*. In contrast, the *cyr1Δ* strains (CR216, SP60-66, SP62-88) and the pseudorevertants derived from them (PR1, PR2, and PR3) failed to give a PCR product, indicating the absence of the catalytic domain as expected. Note that the lanes of the gels are color coded with numbers that correspond to the indicated primer sets shown on the map at the bottom. As a negative control, a set of reactions was performed in the absence of template DNA ("No Template"). The sets of PCR products are separated by lanes containing molecular weight markers (Invitrogen 1KB+ ladder). The positive control product was detected just below the 1000 base pair band as expected.

The following primers were used in this analysis:

Positive Control Primers (primers to *BCY1* open reading frame), expected product is 980 bp.

BCY1-SP0028F: CCACATCTGAATCACGACGA

BCY1-SP0028R: TCCAATGCTTCCACAGTAGC

Primers for *CYR1* Catalytic domain (in the order they appear in the figure). Expected products are 266, 459, 530, and 690 base pairs respectively.

CYR1-set 1 SP0043F: GACAATTGCGAATTACTGGTGG
 SP0043R: GCCGGTTATAACATCTGGTTC

CYR1 set 2 SP0076F: GTGGAGACTCTAATCTCCGT
 SP0076R: CTCTATTGACCATTGGCCCA

CYR1 set 3 SP0077F: CGTCGAGATAAGCAACAAGT
 SP0077R: CTGACAGCAATTTGTCCACC

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CYR1 set 4 SP0078F: ATAGGCAGAAACAAGCTGCC
 SP0078R: TTCCGGCATTTCATTGCCC